ASSESSING ROLE OF MAJOR ALKALOIDS OBTAINED FROM *ARGEMONE MEXICANA* AGAINST THE PLANT PATHOGENIC BACTERIA

A SYNOPSIS

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INTRODUCTION

Medicinal plants are considered as an effective source of both traditional remedies as well as modern medicine. The medicines which are obtained from the plants are much cheaper and effective in comparison with modern medicines (Abhishek *et al.*, 2010). The medicinal plants are used to cure various diseases since ancient times in South Asia (Bhattacharjee, 1998).

Popularity of herbal medicines has increased in recent years. Medicinal utility of plants is due to the presence of a difference of phytoconstituents in the plant tissues, which cast a specific physiological activity on the human body. Some of these constituents are toxic also (Sheeba, 2010). New antimicrobial agents have been discovered from crude extracts derived from plants to eradicate problem of drug resistance (Cordell, 2000; Hemaiswarya *et al.*, 2008; Karaman *et al.*, 2003; Raghavendra *et al.*, 2005).

Natural antimicrobials can be obtained from various plant parts, animal tissues or from microorganisms. These active components can be present in any part of the plant (Gordon and David, 2001; Nair and Chanda, 2004). These plant constituents were proven to be a remedy for a number of human diseases. Many plants are abundant source for their antibacterial, antiseptic and antioxidant effects for their protection from infections. Plants are rich source of bioactive compounds as their secondary metabolites, possessing biological activities (Koduru *et al.*, 2006). The extracts have been used as antimicrobial properties of plant (Del Campo *et al.*, 2000).

Many medicinal plants played a major role against pathogenic microorganisms (Haraguchi *et al.*,1999; Sashikumar *et al.*, 2003). In the past few years, a number of studies have been conducted in different countries to prove such efficiency (Almagboul *et al*.,1985; Artizzu *et al*.,1995; Ikram and Inamul, 1984; Izzo *et al*., 1995; Kubo *et al*., 1993; Shapoval *et al*., 1994; Sousa *et al*., 1991). There are very limited reports on antibacterial activity of plant extracts against plant pathogenic bacteria and no such report on *Argemone mexicana.*

Selection of plant

Botanical description of *Argemone mexicana* :

Botanical name	***Argemone mexicana* Linn.**
Family	**Papaveraceae**
Hindi name	**Pila-dhatura, Satyanashi.**
English name	**Prickly poppy**
Genus	***Argemone***
Species	***mexicana***

Images of plant taken:

Figure 1: *A. mexicana* plant with flowers

Figure 2: Flower of *A. mexicana*

Distribution: *Argemone mexicana* is distributed throughout the tropical and sub-tropical countries including West Africa as an agricultural weed, found in Mexico and now widely naturalised in the United States, India, Nepal, Bangladesh and Ethiopia. In India, it is found in almost every part as wasteland weed. It is invasive in parts of Kenya and Uganda. It is widespread throughout the region and especially on fallow land.

Plant description : *Argemone mexicana* L. belongs to family *Papaveraceae*. It is a prickly annual weed, 60-90 cm height with yellow sap; leaves are simple, sessile and spiny; flowers are large, yellow in colour, between 4 and 6 cms across and six yellow petals; fruits are prickly capsules, oblong-ovoid, opening by 4-6 valves and have numerous small black seeds (Chopra *et al.,* 1986).The plant flowers throughout the year in a tropical climate. This plant is found everywhere by road sides, parks and abandoned fields in India as a weed (Bhalke and Gosavi, 2009).

Significance of selected plant

Traditional uses : The seeds have a cannabis-like effect and the plant parts such as, herb, juice, and flowers, reportedly has potent narcotic effects in many countries. The dried leaves are typically the only parts of the plant available through various Entheogen and Ethnobotanical dealers. This plant is used to treat malaria by traditional healers in Mali (Willcox *et al.*, 2007). The Seri tribe of northern Mexico use the leaves of *Argemone mexicana* to treat kidney disorders. The tea made from the leaves is also said to dispel bad blood that accumulates during birth. The Pima tribe also uses a tea to treat kidney and bladder problems. The Yucatec use the plant to cure gall bladder problems. This plant is used in treatment of muscle pains, cure sleep disorders and treat asthma. In Africa, plant parts are also used for their sedative and tranquilizing properties.

Medicinal uses : It is used as a medicinal plant in several countries. This plant contains alkaloids, similar to those in the opium poppy (*Papaver somniferum*) and so can be used as a mild pain-killer (Chevallier, 1996). Alkaloid such as, Berberine plays a crucial role in the treatment of trachoma and leishmaniasis (Watt and Breyer Brandwijlei, 1962). According to Ayurveda, the plant is used to kill worms and effective in the problem of wound healing (Patil *et al*., 2001). It is helpful to cure diseases like leprosy, inflammations and bilious fever. It possess the analgesic, narcotic, antispasmodic and sedative properties (Emboden,1979; Chevallier,1996). The fresh yellow, milky, seed extract contains protein dissolving substances which are effective in the treatment of warts, cold sores, cutaneous infections, itches and also as emetic, expectorant, demulcent; the seeds and seed oil are employed as a remedy for dysentery, ulcers, asthma and other intestinal affections (Ambasta,1986; Bose *et al.*,1963;

Chopra *et al.*,1956; Prajapati *et al.*,2003; Savithramma *et al.*,2007). This plant is used in dropsy and jaundice (Ambasta,1986; Chopra *et al.*,1956). The seeds are used as an antidote to snake venom in Mexico (Bhattacharjee *et al.,* 2006; Chopra *et al.*,1986). The smokes of the seeds are used to treat toothache problems in India. Symptoms of eating the seeds include limb swelling, nausea, vomiting, diarrhoea, breathlessness and glaucoma. Plant leaves are used to cure malarial fever, ulcers, skin problems, maintaining normal blood circulation and cholesterol level in human body (Albuquerque *et al.*, 2007). The root of this plant is diuretic, alterative, anodyne and used in chronic skin diseases. Its seeds are laxative, purgative, expectorant, digestive and its latex is used against conjunctivitis (Agra *et al.*, 2008). Its latex is used to treat problems like cataract, reddening and itching in the eyes. The root of *Argemone mexicana* is used as anthelmintic. It exhibits antibacterial, antimicrobial, antioxidant and antifungal properties (Mohana *et al*., 2008; Osho and Adetunji, 2010; Shyam Prasad and Dhanapal, 2010; Singh *et al.*, 2009).

Phytochemical constituents: *Argemone mexicana* contains isoquinoline alkaloids. The Isoquinoline alkaloids are found in many plants, including cacti and poppies. There is now a great debate amongst chemists as to whether or not isoquinolines have the potential for psychoactive effects**.** These effects are most likely due to the interesting amalgam of alkaloids present in all parts of this plant with the highest concentration found in a part that is rarely used i.e, roots. The seeds contain two toxic alkaloids, sanguinarine and dihydrosanguinarine. The plant contains alkaloids such as berberine, protopine, sarguinarine, optidine, chelerythevine, etc. The seed oil contains myristic, palmitic, oleic, linoleic acids etc (Jain *et al*., 2012). The leaves contain chemical constituents are flavanoids, sterols, tannins, alkaloids and glycosides (Bhalke *et al*., 2009). Various isoquinoline alkaloids viz. berberine, cryptopine, coptisine, muramine, scoulerine, stylopine, cheilkanthifoline, sanguinarine, chelerytherine, thalifoline, protopine are reported (Gupta *et al.*,1990). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Evans, 2002). Two new protopines agemexicainies A and B and the anti HIV alkaloids 6-acetonyl dihydrochelerythevine which contains various fatty acids in plant were reported by (Chang *et al.,* 2003). *Argemone mexicana* contains

several constituents which include flavanoids, alkaloids, tannins, terpenese and sterols (Quinn-Beatie, 2002).

Selection of plant pathogenic bacteria

Pseudomonas syringae (MTCC 2730), *Xanthomonas campestris* (NCIM 5028), etc., will be used for the study of antibacterial activity. *Pseudomonas syringae* (MTCC 2730) is in top list of bacterial plant pathogens. *P. syringae* (MTCC 2730) continues to cause economically important plant diseases and has a impact on our scientific understanding of microbial pathogenicity. The economic impact of *P. syringae* (MTCC 2730) is increasing with a resurgence of diseases such as, bacterial speck of tomato (pv.tomato) and the emergence bleeding canker of horse-chestnut (pv.aesculi), causes infections of importance worldwide.

Xanthomonas is a phytopathogenic bacteria, which causes the plant diseases like., *Xanthomonas campestris* (NCIM 5028), *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axonopodis* pv. *manihotis* etc. all around the world. The hosts of *Xanthomonas* include 124 monocotyledonous and 268 dicotyledonous plants, among which the rice bacterial blight, cabbage black rot and citrus blight diseases in a range of crops worldwide which cause agricultural production every year. Chemical methods to control economically important blight disease. However, increasing public concern on environmental issues desires that alternative management systems be evolved either to reduce pesticide dependant or naturally occurring compounds be explored to constrain the pathogen attack (Cuthbertson and Murchie, 2005). Pathovars of *Xanthomonas* damage crops and several vegetable (Mandavia *et al*.,1999). This seriously hinders the management of diseases of crops and agriculture products.

In general, most of the studies are directed to see the activity of *Argemone* extracts against a variety of test bacteria namely food-borne pathogens and human pathogenic bacteria (Brahmachari *et al.*, 2013) and in particular not many studies were performed on the antibacterial activity of plant extract against plant pathogenic bacteria. No work is reported on the antibacterial activity of *Argemone mexicana* against plant pathogenic bacteria. Even role of major alkaloids obtained from this plant

against plant pathogenic bacteria has not been reported. Therefore the present study has been designed to assess the role of major alkaloids obtained from *Argemone mexicana* against plant pathogenic bacteria, namely *Pseudomonas syringae* (MTCC 2730) and *Xanthomonas campestris* (NCIM 5028) etc.

OBJECTIVES

The study aims to investigate plant extracts of *Argemone mexicana* against plant pathogenic bacteria with the following objectives:

1. Screening and assessment of antibacterial activity of aqueous and solvent extracts of different plant parts of *A. mexicana* against various plant pathogenic bacteria.
2. Isolation of major alkaloids of *A. mexicana.*
3. In vitro assessment of antibacterial activity of major alkaloids against selected two gram positive and two gram negative strains of plant pathogenic bacteria.
4. To determine minimum inhibitory concentration; MIC levels of extract and alkaloids against selected plant pathogenic bacteria.
5. Characterization of bioactive component with maximum antibacterial activity obtained from the plant extract of *A. mexicana.*

REVIEW OF LITERATURE

Bhattacharjee *et al.*,(2006) evaluated antibacterial activity of *Argemone mexicana* solvent extracts of leaves and seeds against some pathogenic bacteria viz. *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.* Methanol extract showed potent antibacterial effect against the pathogens followed by hot and cold aqueous extract.

Rahman *et al.*,(2009) studied In vitro antibacterial potentiality of *Argemone mexicana* L. (*papaveraceae*) stem extracts against *Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Campylobacter jejuni* and *Vibrio cholera.* The organic extracts showed maximum inhibition against test pathogens. The zones of inhibition were in the range of 10.1 to 21.4mm.

Singh *et al.*,(2009) tested antibacterial activity of seed extracts of *Argemone mexicana* L. on some pathogenic bacterial strains, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The chloroform extract was found to be more potent in comparision with other extracts.

Bhattacharjee *et al.*,(2010) isolated and identified antibacterial components in seed extracts of *Argemone mexicana* L. against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The presence of alkaloid displayed the active fraction (CH3).The CH1 and CH2 fractions did not show inhibitory activity.

Imran *et al*., (2010) showed antifungal activity of *Argemone mexicana* poppy. The protein part was extracted and tested against four different fungal strains viz. *Aspergillus niger, Aspergillus terreus, Aspergillus flavus, Rhizopus stolonifer* etc. A potent antifungal activity was observed at temperature 20’C-25’C, the activity was lost completely at temperature 50’C and above.

Prasad *et al.*,(2010) studied antibacterial and antifungal potentiality of methanolic extract of *Argemone mexicana* leaves against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Aspergillus niger, Fusarium*

moniliforme, Candida albicans and *Mucor plumbeus* were taken, 100µl showed maximum inhibition against all the test pathogens under investigation while, 70µl showed activity against *Staphylococcus aureus, Escherichia coli,* and *Proteus vulgaris* and all the fungi under study.

Singh *et al.,* (2010) investigated four quaternary isoquinoline alkaloids, dehydrocorydalmine, jatrorrhizine, columbamine, and oxyberberine, were isolated from the whole plant of *Argemone mexicana* Linn. (*Papaveraceae*). Their structures were established by spectral evidence.

Abubacker and Ramanathan (2012) studied antibacterial potentiality of leaf extract of *Argemone mexicana* L. (*Papavaraceae*) on pathogenic bacterial strains, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The ethanolic extract showed maximum antibacterial activities against the test bacterial strains followed by hot aqueous extract and cold aqueous extract.

Jain *et al.*,(2012) evaluated antibacterial and antioxidant activity of fruits extract of *Argemone mexicana* Linn. against *Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermis, Shigella flexineri, Bacillus subtilis* and *Escherichia coli*. Results showed that methanolic extract was found to be more effective against gram negative bacteria.

Bhardwaj *et al.*,(2012) made an invitro study of antimicrobial potential of *Argemone mexicana* against *Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and one yeast i.e. *Candida albicans*. Chloroform extract displayed broad spectrum activity.

Saranya *et al.*,(2012) studied invitro antibacterial activity and preliminary phytochemical analysis of leaf extracts of *Argemone mexicana* Linn. Following bacteria were tested, *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Staphylococcus aureus* and *Klebsiella pneumonia.* The antibacterial activity of methanol extracts showed more potent followed by chloroform and petroleum ether extracts against all the bacterial strains.

Gomare and Ghuget (2012) evaluated maximum antimicrobial activity of *Argemone mexicana* using solvent extracts against some pathogenic bacteria. Ethanol and methanol extracts were more potent than aqueous extracts of *A.mexicana*.

Brahmachari *et al.*,(2012) made a critical review on chemical and pharmacological aspects of *Argemone mexicana.*

Bais *et al.*,(2013) evaluated antimicrobial activity of plant leaf *Argemone mexicana*, Eleven clinical isolates of bacteria and fungi were tested *viz. Bacillus subtilis, Staphylococcus aureus, E.coli, Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris, Candida albicans, Candida tropicalis, Aspergillus niger, Aspergillus flavus* and *Aspergillus candidus.* The methanol extracts of *Argemone mexicana* leaf were screened invitro for antibacterial activity and it doesn't show any antibacterial activity against bacteria but it quite sensitive to fungi. It shows remarkable action against *Candida albicans, Aspergillus niger* 24 mm and 22 mm respectively.

Veni and Pushpanathan (2014) investigated antimicrobial and phytochemical screening of *Argemone mexicana* plant extracts against bacteria with gastrointestinal relevance. The ethanolic and methanolic extract of *A.mexicana* showed significant antimicrobial activity in comparision with other four extracts. This revealed the presence of higher content of reducing sugar, flavanoids, tannin, sterol, terpene and alkaloid in all the extracts of *A.mexicana.*

Khade (2015) reported antimicrobial activity and phytochemical screening of *Argemone mexicana* Linn against *E.coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Bacillus cereus.* The ethanolic extract showed potent activity against all the test pathogens. Phytochemical analysis showed the presence of tannins, steroids, flavonoids and alkaloids.

METHODOLOGY

1. Screening and Assessment of antibacterial activity of aqueous and solvent extracts of different plant parts of *Argemone mexicana* against various plant pathogenic bacteria.

1.1) Collection of plant material: Healthy plant samples of *Argemone mexicana* will be collected randomly from the Agra region. The plant will be identified by a taxonomist in the Department of Botany, D.E.I.,Agra. Fresh plant materials will be washed by sterile distilled water, cut plant into small parts and will be dried at room temperature until complete disappearance of moisture in the shade. After drying, powder of plant parts will be prepared with the help of grinding and the obtained powder will be stored in an air tight container at the moisture free space for further study.

1.2) Preparation of plant extracts: The fine powder of plant parts will be extracted successively. All the plant extracts will be filtered using sterile filter paper (Whatman No.1) into a clean conical flask and then the solvents will be subjected to water-bath evaporation and will be utilized for the experiments.

1.2.1) Aqueous extract: The samples of *A.mexicana,* will be weighed and soaked in water in a conical flask stoppered with the use of rubber corks and left undisturbed for 24 hrs, then filtered through whatman filter paper No.1 in a clean conical flask and will be evaporated using water-bath, where the water will be evaporated at its boiling temperature of 100ºC. The standard extracts obtained will be stored in air tight container at 4ºC in refrigerator for further experiments (Akueshi *et al*., 2002).

1.2.2) Solvent extract*:* Different solvents like ethanol, methanol and chloroform will be mixed with powdered plant material separately in Erlenmeyer flask. The respective mixtures will be allowed to stand for 3-5 days in tightly sealed vessels at 40-60ºC, protected from sunlight and mixture will be stirred at 24h interval with a sterile glass rod. This mixture will be filter through Whatman filter paper no.1 and the residue, adjusted to the required concentration. The extracted liquid will be subjected to water bath evaporation at 55ºC and the plant extracts obtained will stored in air tight container in refrigerator at 4ºC for further experiments.

1.3) Maintenance of bacterial cultures:

1.3.1) Procurement of bacteria: Plant pathogenic bacterial strains will be used for determining antibacterial activity of obtained plant extracts of *A.mexicana*. Cultures of plant pathogenic bacteria will be procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh; National Collection of Industrial Microorgainsms (NCIM), Pune or other available sources. Plant pathogens which will be used for study are *Pseudomonas syringae* (MTCC 2730) and *Xanthomonas campestris* (NCIM 5028) etc.

1.3.2) Revival of Pathogen: The collected pathogens will be revived in nutrient broth and stored in nutrient agar slants at 4ºC**.**

1.3.3) Nutrient agar medium (NAM):

Beef extract	**1gm**
Yeast extract	**2gm**
Sodium Chloride	**1gm**
Peptone	**5 gm**
Agar	**20 gm**
Distilled water	**1000 ml**

1.4) Antibacterial assays:

- Agar well diffusion method (Perez *et al*., 1990)
- Paper disc diffusion method (Bauer *et al*., 1996, NCCLS 1993).

1.4.1) Method: Preparation of NAM (Nutrient agar medium). Sterilization of media through autoclave for 15 minute at 121ºC and 15 psi pressure, then will be poured into the sterilized petri plate to a uniform depth of 4mm and allowed to solidify at room temperature. After solidification, the test organisms will be inoculated with the help of a sterile swab soaked in suspension.

Disc diffusion method: The sterile filter paper discs (6mm) containing samples (100µl) will be immersed in plant extracts and placed over the solidified agar in such a way that there is no overlapping of zone of inhibition. Plate will be kept at room temperature for half an hour for diffusion of the sample into the agar media. The petridishes will be incubated at 37ºC for 24hrs.

Agar well diffusion method: Sterilized cotton swabs will be dipped in the bacterial culture in nutrient broth and then spread on the respective media. The agar plates will be punched using a sterile cork borer to form wide wells and the crude extracts (100 µl) will be added into the wells. After the incubation period is over, the zone of inhibition produced by the sample with different organism in different plates will be measured and recorded immediately by scaling.

Different plant parts individually in different solvents and aqueous extracts and whole plant extract will be assayed for antibacterial activity. Control will be (solvent /aqueous solution used in each extract).

2. Isolation of major alkaloids of *Argemone mexicana*

2.1) Test for alkaloids (Oguyemi, 1979 [6])

2.1.1) Mayer's test: Take the filtrate in test tube, 1ml of Mayer's reagent will be added drop by drop. Alkaloid solution forms white yellowish precipitate when added a few drops of Mayer's reagents. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Evans, 2002).

The alcoholic extract will be evaporated to dryness and the residue will be heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture will be filtered and treated with a few drops of Mayer's reagent. Formation of a yellow precipitate will indicate the presence of alkaloids.

2.1.2) Dragendoff's test: Take the filtrate in test tube, 1ml of Dragendoff's reagent will be added drop by drop. Alkaloid solution forms reddish-brown precipitate (Evans, 2002).

2.1.3) Wagner's test: Take the filtrate in test tube, 1ml of Wagner's reagent will be added drop by drop. Alkaloid solution forms reddish-brown precipitate (Evans, 2002).

2.2) Isolation of alkaloids: This will be done according to standard procedures of Trease and Evans (1978), Nuhu *et al*., (2000) with some modifications. Extraction of 1gm of the sample with 30mL of methanol: water (1:1,v/v) mixture. It will be filtered using sterile filter paper (Whatmann No.1) and then filtrate will be subjected to evaporation. The resultant residue will be mixed with 10 mL of 0.0025 M Sulphuric acid and partitioned with ether to remove unwanted materials. The aqueous fraction will be basified with strong ammonia solution and then extracted with excess chloroform to obtain the alkaloidal fraction or separated by filtration. The chloroform extraction will be repeated several times and the bulk extract will be concentrated to dryness. The purity of the substance will be determined by means of the TLC.

2.3) TLC (Thin Layer Chromatography) : Thin layer chromatography will be performed on a sheet of glass, plastic or aluminium foil, which will be coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide or cellulose, i.e., stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture,(i.e.,mobile phase) is drawn up the plate via capillary action because different analytes ascend the TLC plate at different rates, separation is achieved. The methanol, ethanol, aqueous and chloroform extracts of the *A. mexicana* will be subjected to TLC analysis, to find the presence of alkaloids. Precoated TLC plates will be used for the analysis. Separation of alkaloid from the *A.mexicana* will be done using TLC/ HPLC.

3. In vitro assessment of antibacterial activity of major alkaloids against selected two gram positive and two gram negative strains of plant pathogenic bacteria.

3.1) Isolated major alkaloids will be assayed using paper disc and agar well diffusion method as described above.

4. To determine minimum inhibitory concentration; MIC levels of extract and alkaloids against selected plant pathogenic bacteria.

MIC levels of extract and alkaloids against selected plant pathogenic bacteria. Different dilution method will be made to know the minimum requirement of the plant extract against the selected plant pathogenic bacteria. Incubated at 37ºC for each bacterial culture.

5. Charaterization of bioactive component with maximum antibacterial activity obtained from the plant extract of *Argemone mexicana.*

5.1) <u>HPLC (High Performance Liquid chromatography)</u>: The sample of 1gm will be kept overnight for extraction in HPLC grade methanol. This extracts will be sonicated for 20 min in sonicator 20 ul from sonicated extracts will be passed through 0.45 mm filter. Filtrate will be used for HPLC analysis. The HPLC analysis of the sample will be performed according to the method of Sarma *et al.*,(2002).

5.2) <u>Characterization</u> – The isolated alkaloids will be identified using advanced analytical methods like LC-MS. This will be done according to standard procedures of Priya *et al.*,(2013).

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